RNA Biology
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Lecture - 25
RNA Splicing, Export and Stability: Introns in RNA Splicing

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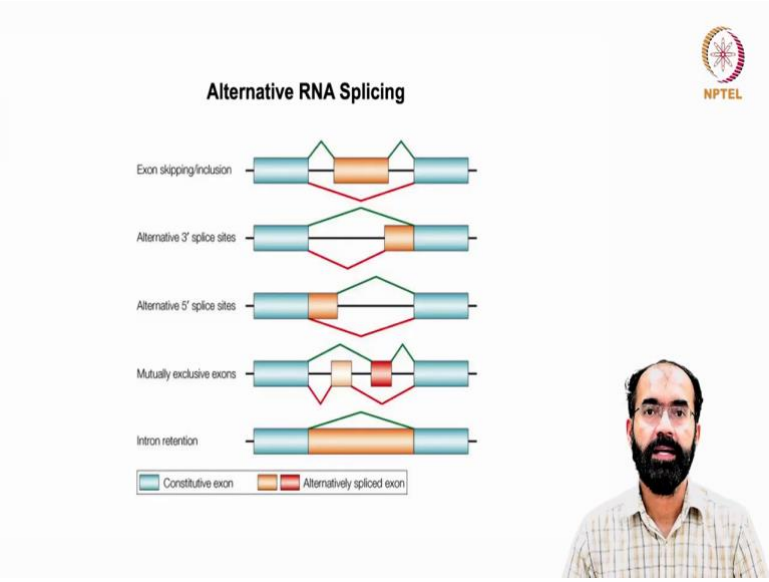

Welcome back to another session of RNA Biology class and we were here studying different possible alternative RNA splicing that can bring in diversity in the transcript. So, we were looking into one-by-one examples one is exon skipping or exon inclusion alternative 3 prime splice site, alternative 5 prime splice sites mutually exclusive exons like that. So, there are different types of variations are possible in when you are considering the alternative splicing.

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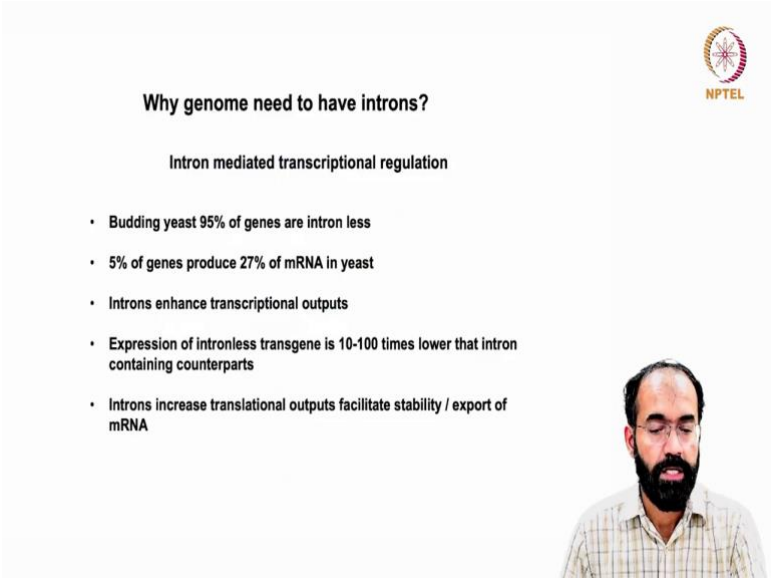

So, again in again, continuing the question of why genomes need to have introns? Introns mediated transcriptional regulation; that means, in budding yeast 95 percent of the genes are intron less. And 5 percent of the genes produce 27 percent of mRNA in yeast. This is something important you should keep in mind how 5 percent of the genes 95 percent of the genes are intron less and 5 percent of the genes are with introns.

And it is important not that 5 percent of the genes produce 27 percent of the mRNA in the yeast and this itself indicate that the diversity is a important possible way that the introns bring in. And introns enhance transcriptional outputs the rate of production of RNA from the gene or from the genetic material is enhanced if introns are there. Expression of intron less trans gene is 10 to 100 times lower than that containing the counterparts.

So, genes can be same whether you include the intron or not can decide whether the transgene you inserted into the genome of an organism express or not. Introns can increase translational outputs to facilitate the stability export of a given mRNA. So, it may sound little strange that how come introns can influence the translational output? When the introns are completely missing from the mature mRNA? So, we can see them case by case as we proceed towards those sections.

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And intron can mediate transcriptional regulation we have seen it and this is something called splicing dependent; that means, the transcriptional regulation how introns will mediate is at the stage of splicing. The U1 snRNA the first snRNA that binds on to the exon intron boundary ok, which binds to the 5 prime splice site interacts with the general transcription factor TFIIH.

You may remember TFIIH is one of the subunits one of the transcription factors of RNA polymerase 2 which has got the helicase activity. So, U1 snRNA is normally is important in the splicing. Remember when the RNA is come out soon after the transcription begins you have the capping event that takes place and also it binds U1 snRNA binds to the 5 prime splice sites that can also interact with the transcription factor TFIIH which is bound down to the DNA.

So, the RNA is just started its transcription and it is also important to note that many of the genes the first exon is pretty small it has got the untranslated region and may be one or two amino acids or may be less than 10 amino acids of that actual protein. So, untranslated region will be there in the first exon. And some genes are there they the first exon do not have any coding region it is purely part of the untranslated region. It is the second exon onwards there will be any translation.

So, why I am saying this? Because first exon can be very very small. Because of which the exon intron boundary will be much earlier because if the exon is say 2000 base let us

assume the first exon is 2000 base, then the RNA has to transcribe that much long before the U1 snRNA binds to the exon intron boundary right because RNA transcription is not completed.

But if the first exon is small then it can U1 snRNA binds and then it can interact with the TFIIH because it is the newly formed RNA still in the vicinity of the DNA itself it has not come out very far. And then another regulation is splicing independent what is that? Presence of enhancer and promoter element is seen in the introns.

Normally, when you say enhancer or gene regulator means it can attract some transcription factors. Many genes the first intron of many genes can attract factors that can influence the transcritability of the gene. So, if you are making a transgenic animal, it is often advisable to include the first intron also somewhere in the promoter region it need not be exactly in the same place if it is in the exactly in the same place that is great.

But at least if you include somewhere in the regulatory elements if you include the first intron of that gene also that will enhance the transgene expression. So, that is why the introns do contain the introns do contain a lot of sites binding sites which attracts the transcription factors.

Transcription factors here we are not referring to the general transcription factor like RNA polymerase two etcetera, but it is tissue specific transcription factor that allows whether or not a given gene should be expressed in a tissue or how efficiently it should be expressed etcetera that will be decided by the tissue specific transcription factors.

That is why? Liver tissue produce only liver gene it do not produce kidney genes or brain genes. Same way brain cells produce brain specific genes it do not produce liver genes or you know intestine genes. So, this is called a tissue specificity. So, to a great extent the introns of those genes which are expressed in that particular tissue have sequences that will attract the protein factors.

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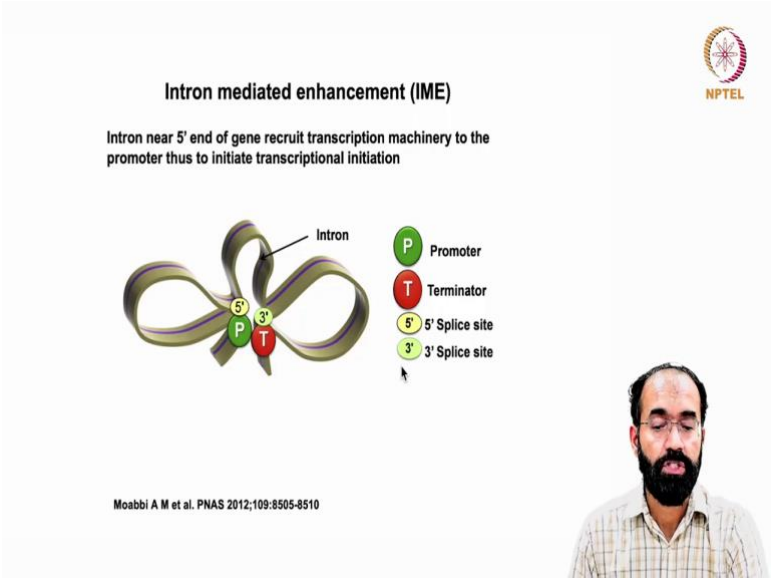

So, intron mediated enhancement let us see how does it perform the show? So, in this cartoon you can see there is a promoter element and then end of the gene terminator element and then you have got 5 prime and 3 prime splice sites. So, this is the intron supposedly removed.

So, you have a promoter this is the exon this is the intron another exon and the termination it is a one of the simplistic example because it is a cartoon it has got 2 exons and 1 intons. So, the intron near the 5-prime end of the gene recruit transcription machinery to the promoter thus to initiate the transcriptional initiation.

That means, promoter is here and the intron brings in some of the transcription factors and it can bring in this machinery close to the promoter site by looping and that can facilitate the transcribability or the kick starting of the or the rate of production of the RNA can be influenced.

So, intron near the 5-prime end of the gene recruit transcription machinery to the promoter to initiate the transcriptional initiation. So, this is what you should keep in mind that you can facilitate the transcribability or the transcription.

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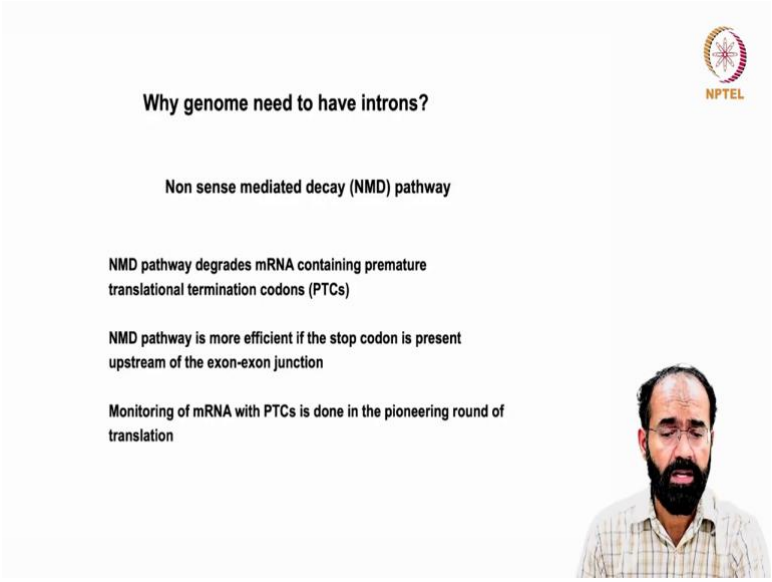

Why genome need to have introns? And we saw one such example is the nonsense mediated decay ok. We will see in detail that how does it work. NMD nonsense mediated decay in short form we can call it as NMD pathway degrades mRNA containing premature translation termination codon.

We have seen some example like apolipoprotein be we have saw that you know introduction of UAA 3 stop codons are the UAA, UAG, UGA these are the 3 stop codons of the 64 stop 64 codons 3 of them are stop codons. So, NMD pathway degrades those mRNA that contains premature translation termination codon. Now, the question is who will tell which is mature or premature? This is what you should keep in mind.

If there is a premature stop codon is there then that RNA should be marked for degradation otherwise what will happen it will keep on producing some protein a incomplete protein which is useless to the cell. So, this RNA itself should be degraded. So, that is how it should work. But how is it doing? NMD pathway is more efficient if this stop codon is present upstream of the exon-exon junction.

Remember, so far, we have been talking about exon, intron boundary. Here we are talking about exon-exon junction in a mature mRNA. So, say if mature mRNA has got three exon; exon 1, exon 2, exon 3 it is supposedly having two exon-exon junction. One junction is exon 1 and exon 2 and the second junction is between exon 2 and exon 3. So, in the mature mRNA some identity should be there that where was the exon boundary.

So, here the NMD pathway efficiently works if the stop codon is present upstream of the exon-exon junction. Keep this in mind how does it work we will see? So, monitoring of mRNA with termination codons is done in the pioneering round of translation; that means, when the RNA is translated for the first time.

Of course, this defective stop codon defective protein is formed in the one round it is formed. Then the system has to recognize ok there is a stop codon this RNA did not encounter or this RNA did not utilize the actual stop codon rather it utilized a defective or a premature stop codon and this is detected in the first round.

So, what it indicates this RNA when it is formed freshly from this post splicing event it retains a something which marks the exon-exon boundary? So, that is what actually happens? This exon-exon boundary is bound with some factors, some proteins which will give message to the translational machinery through merely by physical interaction that is why you are able to detect whether it is upstream of a exon-exon boundary.

If it is right in the middle, it is nowhere close to an exon-exon boundary the mutation persists because there can be some other way of degrading it, but nonsense mediated decay will not come into picture. We will see that more in detail with the example.

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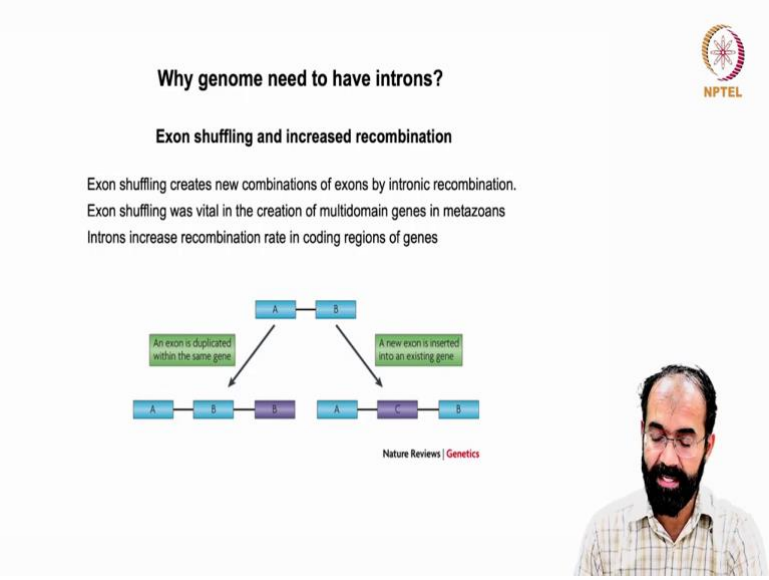

So, exon shuffling has increased the rate of recombination. So, exon shuffling we saw that one way of introducing complexity. So, exon shuffling creates new combinations of

exon by intronic recombination. Exon shuffling was vital in the creation of multi domain genes in metazoan. Metazoan means multi cellular organisms.

So, introns increase the recombination rate in the coding region of the gene. Let us see some example say a gene that has got A and B two exons are there and an exon is duplicated within the same gene. So, the we saw in one of the earlier class evolution by gene duplication by NIOS book. So, those who are interested can read about that and if a gene giving rise to this A B is making a copy of itself A B.

But what if this B is duplicated? Because of a replication error or a guided decision. Especially if there are plenty of repeats are there like intron mediated, interval sequences are there lot of repeats are there is always a problem of making an extra copy. And A, B and you have got a one more additional B that is present. And this gene need not necessarily suffer because A, B is perfectly there and this extra B is you know like you are going to write an exam you have one good pen.

Is there anything wrong if you have one more pen if your one good pen is working perfectly fine you will not even touch the second pen right. But so having the second pen will allow you some luxury of using it for some other purpose. So, you can use it as if it is a heavy fountain pen you can use it as a paper weight in the exam hall. So, that your other papers will not fly off question paper will not fly off like that.

So, this extra exon can acquire some new function by changes of course, B is not that is why it is given in a different color. It is not identical to B after not to start with it was identical to B, but slowly it can acquire changes and this can give rise to a novel additional function. Another option is A, B is made a copy of itself A B is very much there, but right in the middle you had the intronic sequence a new exon is inserted into the existing gene via the recombination.

Who provided the atmosphere for recombination the intron provided the atmosphere for recombination. Because exon also can provide because there is no difference between exon and intron unless you are bringing into a mRNA context, but here the recombination happened in the intronic sequence will be viable.

Because intron can tolerate changes tolerate variations because it is first of all its larger in length larger in size. Whereas, if this recombination has happened in the exon region

there is a very good possibility that the organism may suffer because of this recombination the gene's function may get disrupted.

So, it will be as good as not having this gene at all. So, introns become handy to introduce these new exons or some changes etcetera. If the introns were not there this evolution could have been problematic or not possible.

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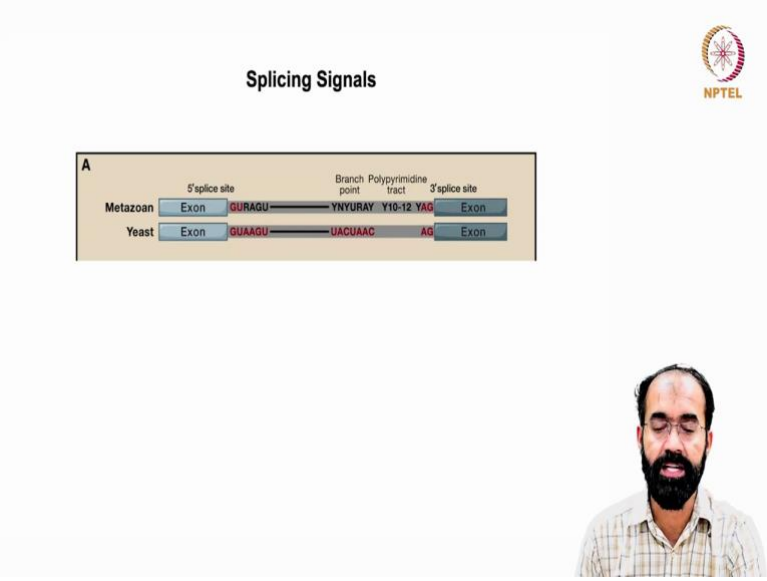

So, now let us see how introns and their splicing signals are influencing the further complexity. So, in a metazoan if you see you have an exon and another exon and this is the five prime splice site and you have this GU AG rules are maintained and you have the branch point and you have the polypyrimidine tract.

And in yeast also you have both of them there can be slight variation, but GU A G rule is followed strictly and this is the typical splicing signal.

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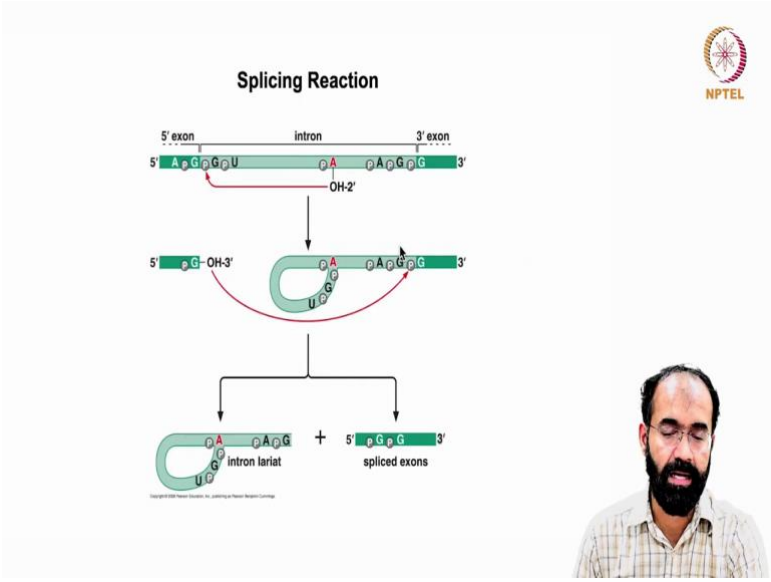

Let us see how the splicing reaction takes place we have seen it we are just quickly revisiting and whenever there is an alteration that can be emphasized. This branch point does the first round of transesterification reaction and then this release exon does the second round of transesterification reaction. So, that this exon fused with this exon and you have end up getting the spliced exons and the intron lariat that will be released this is the typical splicing reaction we have seen it.

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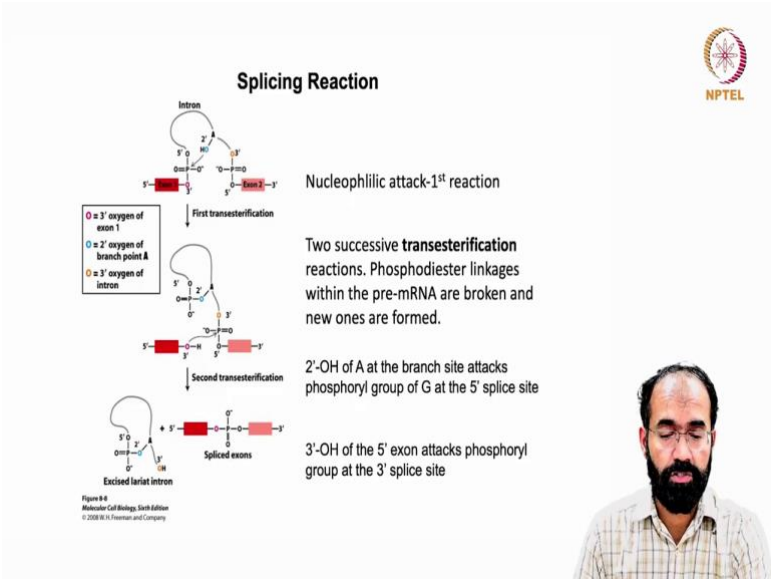

And the splicing reaction also we have seen it the nucleophilic attack of the 1st reaction and two successive transesterification reaction and the phosphodiester linkage within the pre-mRNA broken and the new ones are formed. The 2 prime OH of an A always remember it is the 2 prime OH of the branch point site A or adenine at the branch site attacks the phosphoryl group at the G in the 5 prime splice site.

We know its start with intron is starting with the G and the second base is U. The 3 prime OH of the 5 prime exon attacks phosphoryl group at the 3 prime splice site.

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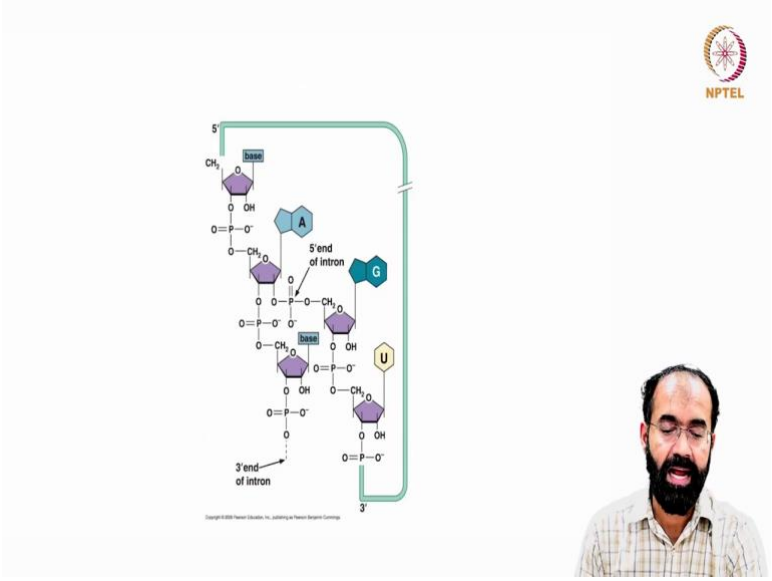

So, this is the second event that is the second transesterification reaction and this is exactly what is that bonding that has happened you have a base and you have a the pentose sugar and its continuing and this is exactly the 3 prime end of the intron and this is the 5 prime end of the intron at molecular level. So, this is basically shown for extra clarity and extra information. So, this is the way the lariat looks like in the intron once it is excised out.

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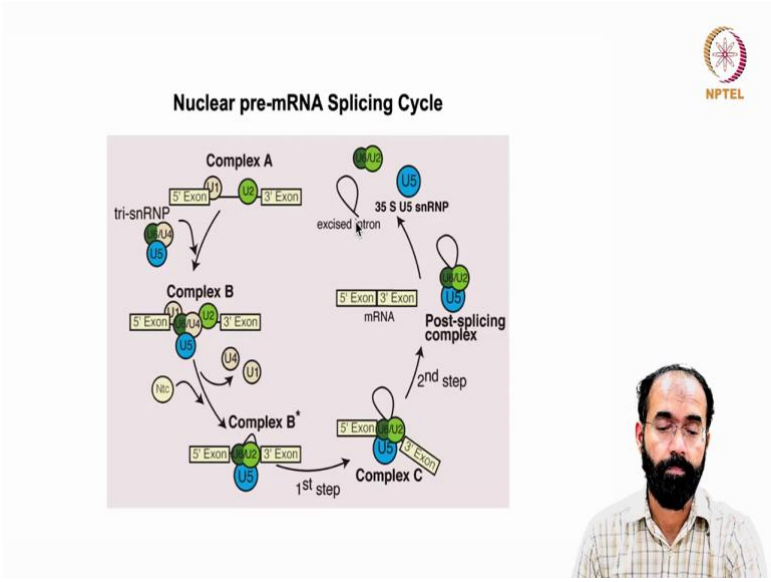

So, now quickly let us go into the stepwise manner in which the RNA splicing is taking place that nuclear pre-mRNA splicing cycle. It forms different complexes different complex A complex B complex B star and it completes one cycle and complex C and it completes one cycle. Complex A the first step U1 and U2 snRNA they come and bind and remember this snRNAs also have proteins.

So, we call it snRNPs. So, they come and pair and U1 binds to the exon intron boundary U2 binds to the branch point. Once this is bound then you have got a tri-snRNP that comes in U6, U4, U5 joins and it forms the complex B. And this complex Bs role is to bring in the branch point close to the 5 prime splice site that is what happens the role of the complex B.

And because the complex B that is assembled then you will have the release of U1, U4, snRNP's and this will have the joining once they release the joining of NTC a new protein joins the complex. And because of this the U6, U2 and U5 stay back because U2 is important because that is bind into the branch point and this is the 1st step. And you have a intermediate stage of complex B star which is quite short lived and you have the 1st step of the splicing takes place between the branch point and the 5 prime splice site.

So, U6, U2 and U5 exist and then comes the 2nd step. 2nd step is what the 3 prime OH of the first exon does the second transesterification reaction onto the 5-prime end of the second exon. And the 2nd step once it is completed you have the clean spliced RNA

came out and you have the 2nd step completed as a result RNA comes out and you have the U6, U2, and U5 which is called post splicing complex that will be released along with the intron lariat.

And once this complex is released it further disassembled so, that the excised intron in the form of nucleic acid it will be released and U6, U2 released as a pair and U5 is released as a separate pair. And this acting as a 35S U5 snRNP and these molecules are available for next round of reaction as you saw here, they will join the reaction further. But this U6 and U2 also will separate eventually and the cycle continues complex A, complex B, complex B star, complex C and then post-splicing complex.

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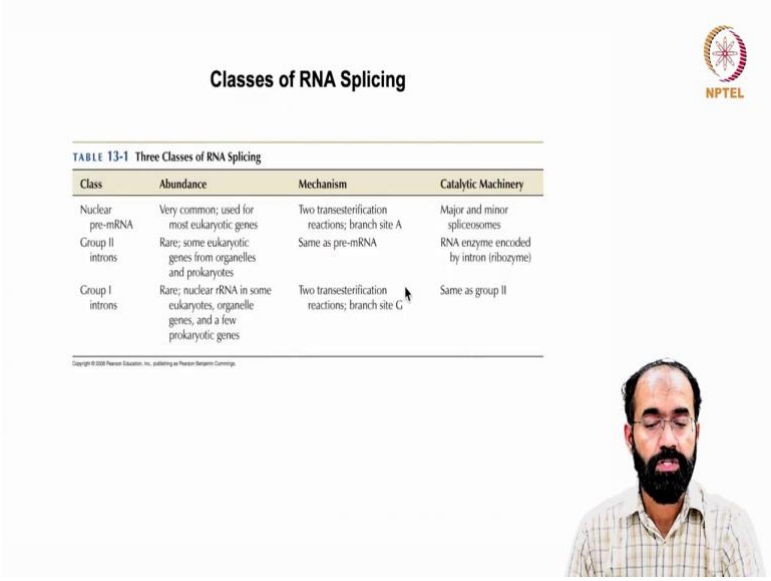

So, there are different classes of RNA splicing we have seen it and this is a revisiting that nuclear pre mRNA is one important class that is very common used for most of the eukaryotic genes and it contains two transesterification reactions and the branch point is branch A and its major and minor spliceosomes are there. We have seen so far is the major one and minor one we will see in a short while there is also another category called minor spliceosome.

And group II and group I intron we saw from the ribozyme stage itself they are not common they are rare some eukaryotic genes from organelles and prokaryotes. Organelles means mitochondria and in plants and animals and plastids in plants like chloroplast etcetera. And they functioning is same as the pre-mRNA to

transesterification reaction and it is an RNA enzyme encoded by intron that is we call it as ribozyme and no ATP is needed; whereas, the nuclear pre-mRNA splicing require ATP.

And group I introns they are rare and nuclear ribosomal RNA in some eukaryotes perform this and or some of the organelle genes and few of the prokaryotic genes also do the same thing. And two transesterification reactions and this happens at branch site G there is no which is this G is externally recruited.

There is no endogenous A present in group I intron that is recruited and because of the special pocket this G has got. So, this we discussed in detail in the previous class and the outcome is same as that of group II the machinery is same as that of group II introns.

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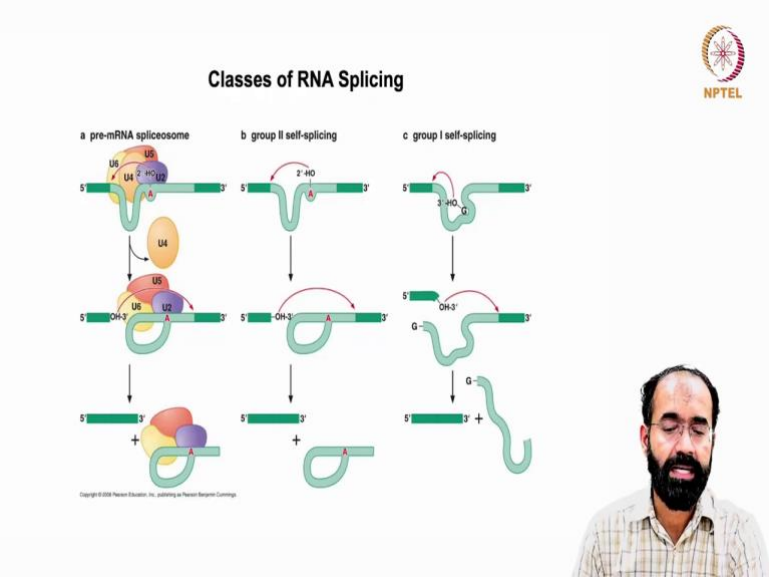

So, if you look into the different classes of RNA splicing the same thing what we discussed is seen in a pictorial manner that pre-mRNA spliceosome you have U1, U2 snRNA and this is the branch point first transesterification second transesterification and spliced exons and the post splicing complex with the intron will be released this is a group II.

Same as that of group I same as that of the pre-mRNA splicing except that there is no spliceosomeal machinery needed there is a branch point a 2 prime oh participate first transesterification second transesterification and the same outcome no dependency on the

snRNA. And the group I is an externally recruited G does the job and remaining it will not create a lariat as you saw in these two cases because it is an external G and G remains free floating attached onto the end. So, this is the only difference.

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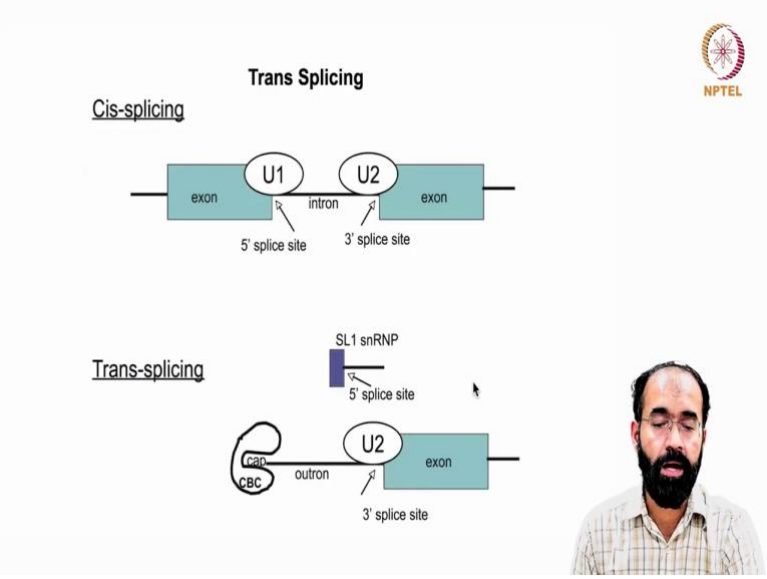

Now, when you look into another variety of splicing that is called trans splicing. So, far we saw cis splicing; cis splicing means like you are scratching your face you are scratching your nose that is cis splicing you are scratching your friends back that is called trans. So, simplistic form this is the difference between cis and trans. So, trans splicing what it indicates someone else is coming from outside to contribute or remove some part. So, that is what you should understand.

So, far we saw group 1 group 2 or pre-mRNA splicing everything is cis because you are doing within you are doing yourself. Even some external factors are coming, but RNA part is same, but here in trans RNA part is come from elsewhere. So, normal case we know U1 and U2 you know 5 prime splice site 3 prime splice site everything is happening perfect everything is happening perfectly fine. Whereas in trans splicing what is happening is that it do not have it do not have this proper machinery required.

It is having an exon, but and also the U2 is binding to the branch site it can bind etcetera. But somehow it is lacking the support from the this is the RNA cap everything is there and there is no exon upstream of that and because of that we do not call this region as intron rather we call it as outron. How is it working let us quickly see it?

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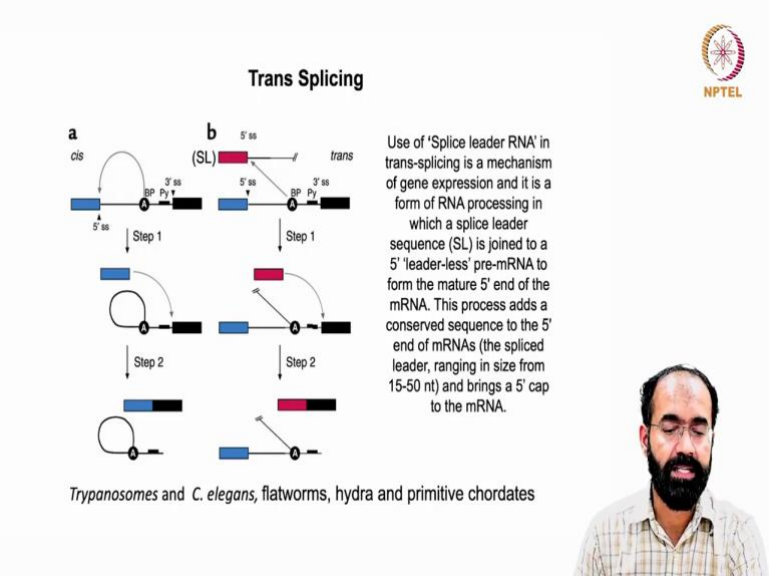

The Trans splicing even is quite interesting. So, normal case is the A it branches 1st step and the 2nd step and you have the normal splicing. In Trans splicing what happens this A instead of going here it goes into the external RNA that has come out and it pairs there and because of this now this particular exon is doing the second transesterification reaction. And now this external exon it became part of this exon.

So, many a times it becomes useful for those genes like you saw in the previous slide here that it does not have the first exon. So, it can contribute to the first exon. And in this case first exon is there, but you do not want the first exon you wanted a variety you wanted diversity. So, this newly formed exon now fuses and it brings in a diverse exon into a RNA which was never there during its transcription.

So, use of splice leader RNA in Trans splicing is a mechanism of gene expression that is a form of RNA processing while which the splice leader sequence SL what you saw here this splice leader sequence which is shown in pink color is joined to the 5 prime leaderless pre-mRNA to form the mature 5 prime end of the mRNA.

So, that it can process further downstream which is lacking in this actual mRNA. This process adds a conserved sequence to the 5-prime end of the mRNA this we call it as the spliced leader and this can have the size ranging from around 15 to 50 nucleotide and brings to the 5-prime cap to the mRNA. And we will see more in detail about the

relevance of trans splicing in the next class how it becomes a savior and helpful in many cases. And I am ending the class now.

Thank you.